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Does synergistic co-activation affect the extent of epimuscular myofascial force transmission between rat ankle plantar-flexors?

Based on:

Tijs C, van Dieën JH, Baan GC, Maas H (submitted). Does synergistic co-activation affect the extent of epimuscular myofascial force transmission between rat ankle plantar-flexors?

Abstract

Force transmission between rat ankle plantar-flexors has been found for physiological lengths and relative positions of the involved muscles, but only if all muscles were maximally activated. The aims of this study were to assess intermuscular mechanical interactions between ankle plantar-flexors during (i) fully passive conditions, (ii) excitation of soleus (SO), (iii) excitation of lateral gastrocnemius (LG), and (iv) co-activation of SO and LG (SO&LG). In addition, the consequences of such mechanical interaction for Achilles tendon force were investigated in the condition that only SO was excited. We assessed effects of proximal lengthening of LG and plantaris (PL) muscles (i.e. simulating knee extension) on forces exerted at the distal SO tendon (F_{SO}) and distal LG-PL tendon ($F_{LG\text{-}PL}$) of the rat. F_{SO} and $F_{LG\text{-}PL}$ in response to SO excitation were summed to obtain active Achilles tendon force (F_{AT}). LG-PL lengthening increased F_{SO} to a larger extent during LG excitation (0.016 N) than during fully passive conditions (0.005 N). Change in F_{SO} due to LG-PL lengthening was lower during SO only excitation (0.034 N) than during SO&LG excitation (0.061 N). For the condition in which only SO was excited, the increase in F_{AT} on LG-PL lengthening was smaller than that of active F_{SO}. This study showed that epimuscular myofascial force transmission between rat ankle plantar-flexors is enhanced by muscle activation, but that the magnitude of this interaction was limited and only partially reflected in forces exerted at the Achilles tendon.

Introduction

Numerous animal studies have reported clear evidence that muscle force can be transmitted to the skeleton via epimuscular myofascial connections (Huijing, 2009; Maas & Sandercock, 2010). The extent of epimuscular myofascial force transmission is dependent on the position of a muscle relative to surrounding structures (Maas *et al.*, 2004; Meijer *et al.*, 2006; Rijkelijkhuizen *et al.*, 2007; Huijing & Baan, 2008). More recently, intermuscular interaction was assessed by imposing exclusively physiological muscle-tendon unit (MTU) lengths and relative positions (Bernabei *et al.*, 2015). Proximal lengthening of active lateral gastrocnemius (LG) and plantaris (PL) muscles (i.e. simulating knee extension) increased the force exerted at the distal tendon of the soleus (SO) muscle significantly (by 12%). This indicates that myofascial linkages can be mechanically relevant within physiological MTU lengths and relative positions.

In other studies using a more intact hindlimb and in which tendons were not disrupted from the skeleton, the mechanical relevance of intermuscular connections has been challenged (Chapter 4; Maas & Sandercock, 2008; Tijs *et al.*, 2015*a*). The MTU length of bi- and poly-articular muscles was changed at their origin, by imposing changes in knee joint angle, while the MTU length of the synergistic mono-articular ankle muscles was kept constant by testing at a constant ankle angle. In those studies, the ankle moment exerted on excitation of the mono-articular muscle was not significantly affected by knee angle, if the synergistic muscles were kept in a passive state. Because the hindlimb was kept intact as much as possible, only the net moment of each muscle at the ankle joint could be assessed, but no information was available about the forces exerted at the individual tendons. Therefore, epimuscular myofascial force transmission per se could not be excluded.

The contradicting results of the above described studies may be explained by differences in experimental conditions. Firstly, in an intact hindlimb ankle plantar-flexion muscles merge distally into the Achilles tendon (Maas & Sandercock, 2008; Tijs *et al.*, 2015*a*). Although forces can be transmitted between these muscles, all force is then transmitted to the skeleton via the common Achilles tendon. As a consequence, any epimuscular myofascial force transmission may not be reflected in the moments exerted at the ankle (Tijs *et al.*, 2015*a*). However, similar results were found for the muscles in the anterior crural compartment, which do not share a

tendon distally (Chapter 4). Secondly, the level of muscle activation and number of simultaneously excited muscles differed between the studies. While mono-articular muscles were excited maximally, synergistic muscles were either passive, partially active (Chapter 4; Maas & Sandercock, 2008; Tijs *et al.*, 2015*a*) or maximally (Bernabei *et al.*, 2015) active. As hypothesized earlier (Maas & Sandercock, 2008), muscle activation may increase the stiffness of epimuscular myofascial connections, thereby, enhancing the extent of mechanical interaction between muscles.

The primary aim of the present study was to assess effects of various levels of SO and LG muscle activation on epimuscular myofascial force transmission between rat ankle plantar-flexors for physiological MTU lengths and relative positions of LG and PL muscles. Effects of LG-PL MTU length on forces exerted at the distal tendon of SO were used as indications of such force transmission. A secondary aim was to assess the consequences of epimuscular myofascial force transmission on the forces exerted at the common Achilles tendon.

Materials & Methods

Animals

Experiments were performed on ten male Wistar rats (body mass 312.9±17.7 g, mean±SD). All procedures were in agreement with the guidelines and regulations concerning animal welfare and experimentation set forth by Dutch law, and approved by the Committee on Ethics of Animal Experimentation at the Vrije Universiteit Amsterdam (Permit Number: FBW 12-01).

According to standard procedures in our laboratory (Maas *et al.*, 2001) the animals were deeply anesthetized by intraperitoneally injected urethane (1.2 ml/100g body mass). If necessary, additional doses were given to suppress any reflexes. During surgery and data collection, body temperature was maintained at approximately 37°C. To prevent dehydration of exposed tissues, a saline solution was applied regularly. At the end of the measurements, the animals were euthanized with an overdose of intracardially-injected pentobarbital sodium followed by a double-sided pneumothorax.

Surgery

The surgical procedures have been described in more detail elsewhere (Bernabei *et al.*, 2015) and will, therefore, be described only briefly here. The posterior crural compartment of the right hindlimb was exposed by removing the skin and biceps femoris muscle and the femur was exposed to allow attachment of a metal clamp. The medial gastrocnemius was removed fully without damaging muscle fibers of LG. All structures surrounding the SO, PL, LG muscle group were cut, but myofascial connections between their muscle bellies were left intact.

The hindlimb was positioned at ankle and knee angles of 90°. Markers were placed on the lateral collateral ligament and distal tendon of the peroneus muscle, and served as proximal and distal reference markers, respectively. Markers on the distal SO tendon, distal LG tendon and proximal LG aponeurosis were placed such that they were parallel to the position of the reference makers. As a result, any changes in SO and LG marker positions could be expressed relative to the reference position (L_{REF}) at 90° ankle and knee angles.

LG and SO tendons merge distally into the Achilles tendon before inserting on the calcaneus. To measure individual tendon forces, these tendons were separated. The distal SO tendon was cut and connected, using Kevlar thread and a metal rod, to a force transducer (ALPHA load beam transducer, 25N maximum capacity, max output error <0.1%, compliance 0.0162mm/N; BLH Electronics Inc., Toronto, Canada). Distal LG and PL tendons were tied together (LG-PL complex) and separated from the skeleton by cutting a small piece from the calcaneus. The proximal LG-PL tendon was separated by cutting a small bone fragment from the lateral epicondyle. Both tendons were attached to force transducers (Z6 bending beam load cell, 50N maximum capacity, max output error <0.1%, compliance 0.0048mm/N; HBM, Darmstadt, Germany) using Kevlar thread and metal rods. The three force transducers were aligned to the muscles' lines of pull.

To excite SO, the sciatic nerve was partly dissected free for placement of a cuff electrode. In addition, all branches distally to the cuff electrode were cut, except the branch innervating SO muscle (Maas & Sandercock, 2008; Tijs *et al.*, 2014). At the end of the experiment, it was checked whether indeed only SO muscle was excited. In five animals, an additional branch to PL was found at the SO-PL muscle belly interface and, hence, SO was not excited exclusively. These animals were excluded

from the analysis. To excite LG, bipolar intramuscular wire electrodes were inserted near a motor endplate located in the proximal region of the muscle (De Ruiter *et al.*, 1995; Prodanov *et al.*, 2005).

Experimental protocol

SO muscle was excited by supramaximal stimulation of the sciatic nerve (amplitude: 0.4-0.5 mA, frequency: 100 Hz, pulse width: 100 μs) via the bipolar cuff electrode connected to a constant current source (Digitimer DS3, Digitimer Ltd., Hertfordshire, England). LG muscle was excited partially via the intramuscular electrodes (amplitude: 0.8-3.0 mA, frequency: 100 Hz, pulse width: 100 μs). Because only one LG compartment was stimulated, not all muscle fibers were excited. At the highest length tested (L_{REF}+3mm, see below), LG excitation yielded a force of approximately 3N. For the same length, but during maximal excitation of the LG-PL complex via nerve stimulation, a force of approximately 14 N was found (Bernabei *et al.*, 2015). Because the ratio of maximal forces exerted by LG and PL is approximately 3:1 (Johnson *et al.*, 2011), we estimated that 10.8 N was exerted by LG and 3.2 N by PL in the study of Bernabei et al. (2015). Based on these data, we estimated that approximately 28% of the LG fibers were excited in the present study.

The distal markers of SO and LG were kept at reference positions (L_{REF}, see above). Thus, SO was kept at a constant MTU length corresponding to a 90° ankle angle. The MTU length of the LG-PL complex was changed proximally (i.e. simulating knee extension) from -3mm to +3mm relative to L_{REF} with increments of 1mm. These length changes correspond to knee joint angles of approximately 45° and 130°, respectively (Bernabei *et al.*, 2015).

For each LG-PL length, four combinations of SO and LG muscle activation levels were applied: passive conditions of SO and LG, separate excitation of either SO or LG, and simultaneous excitation of SO and LG (SO&LG).

Data analysis

Forces exerted at the distal SO tendon (F_{SO}) and distal LG-PL tendons ($F_{LG\text{-}PL}$) were assessed before (passive force) and during SO, LG and SO&LG excitation (total force). For separate SO and LG excitation and for SO&LG excitation, mean total force was assessed as the mean of the total force output of the last 50 ms of tetanic

stimulation.

During SO only excitation, an estimate of active Achilles tendon force (F_{AT}) was calculated. For this, passive F_{SO} and F_{LG-PL} were subtracted from total F_{SO} and F_{LG-PL}, respectively. Subsequently, these forces in response to SO excitation were summed. Active forces were used in order to exclude effects of LG-PL proximal lengthening on distal passive forces. Also, this analysis was performed for the condition with SO excitation only, to avoid effects of LG-PL proximal lengthening on active LG forces.

Statistics

Two-way repeated measures ANOVAs (SPSS 20, IBM, USA) with 'LG-PL length' and 'muscle activation' as fixed factors and F_{SO} as dependent variable were performed for the combination of passive SO with either active or passive LG and for the combination of active SO with either passive or active LG. In case of significant interaction effects, one-way repeated measures ANOVAs were used to test for effects of 'LG-PL length' on F_{SO} for the passive and active muscle conditions, separately. To assess the consequences of mechanical interaction for Achilles tendon force, a two-way repeated measures ANOVA with 'LG-PL length' and 'tendon' (SO, AT) as fixed factors and force output as dependent variable was performed. Given the fact that F_{AT} was estimated as the sum of F_{SO} and F_{LG-PL}, only two tendon forces were considered in this analysis. In case of a significant interaction effect, one-way repeated measures ANOVAs were used to test for effects of 'LG-PL length' on F_{SO}, F_{LG-PL} and F_{AT}, separately. Greenhouse Geisser correction was used if the assumption of sphericity was violated. Level of significance was set at $p \leq 0.05$.

Results

Intermuscular mechanical interaction between passive and active SO and LG

ANOVA indicated a main effect of partial LG excitation (p=0.021), a main effect of LG-PL length (p=0.009) and an interaction effect between LG excitation and LG-PL length (p=0.017) on F_{SO}. For passive muscle conditions, F_{SO} increased from 0.003±0.002 N at L_{REF}-3mm to 0.008±0.006 N at L_{REF}+3mm (p=0.039, Fig. 6.1A), which is a 123.7% change expressed relative to the force exerted at L_{REF}. For LG excitation only, F_{SO} increased from 0.007±0.005 N at L_{REF}-3mm to 0.023±0.012 N at L_{REF}+3mm (p=0.009), which is an increase of 116.6% relative to the force exerted

at L_{REF}.

For active SO conditions (Fig. 6.1B), ANOVA indicated a main effect of LG-PL length (p=0.001) and an interaction effect between SO&LG excitation and LG-PL length (p=0.002) on F_{SO}. However, no main effect of SO&LG excitation on F_{SO} was found (p=0.053). For SO excitation only, F_{SO} ranged from 1.34±0.17 N at L_{REF}-2mm to 1.38±0.16 N at L_{REF}+3mm (p=0.006, Fig. 6.1B), which is a 2.4% change expressed relative to the force exerted at L_{REF}. During SO&LG excitation, F_{SO} increased from 1.38±0.13 N at L_{REF}-3mm to 1.44±0.11 N at L_{REF}+3mm (p=0.001), which is a change of 4.3% relative to the force exerted at L_{REF}. These results indicate that mechanical interaction between ankle plantar-flexors was dependent on the level of muscle activation.

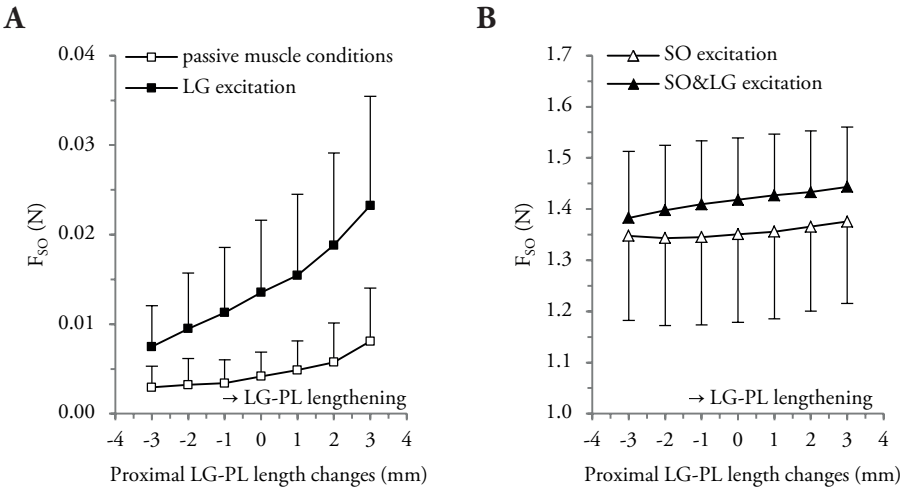

Figure 6.1. Effects of LG-PL length changes on forces exerted at the distal tendon of SO. (A) Distal SO tendon forces for passive conditions of SO and LG muscles (□) and for active conditions of LG only (■). (B) Distal SO tendon forces for active conditions of SO only (Δ) and for active conditions of both SO and LG (▲). Means±SD are shown (n = 5).

Effects of intermuscular mechanical interaction on Achilles tendon forces

Effects of intermuscular mechanical interaction on F_{AT} were studied for SO excitation only. ANOVA indicated significantly higher active F_{AT} than active F_{SO} (p=0.028, Fig. 6.2). $F_{LG\text{-}PL}$ was comparable to the difference between F_{AT} and F_{SO} found, despite the fact that LG-PL muscle fibers were not excited. ANOVA also indicated a main effect of LG-PL length (p=0.007) and an interaction effect between tendon (F_{AT}

vs. F_{SO}) and LG-PL length (p=0.003). Active F_{SO} ranged from 1.34±0.17 N at L_{REF}-2mm to 1.37±0.16 N at L_{REF}+3mm (p=0.024), which is 2.0% relative to the force exerted at L_{REF}. Additional F_{LG-PL} ranged from 0.10±0.07 N at L_{REF}-1mm to 0.08±0.05 N at L_{REF}+3mm (p=0.03), which is -20.1% relative to the force exerted at L_{REF}. Effects of LG-PL length on calculated active F_{AT} (0.8% relative to the force exerted at L_{REF}, Fig. 6.2) were lower than those on active F_{SO}, although the increase in active force (from 1.44±0.11 N at L_{REF}-3mm to 1.45±0.11 N at L_{REF}+3mm) was still significant (p=0.024). These results indicate that epimuscular myofascial force transmission between ankle plantar-flexion muscles is only partially reflected in the Achilles tendon force, due to opposite effects on forces exerted at the distal tendons of both synergists.

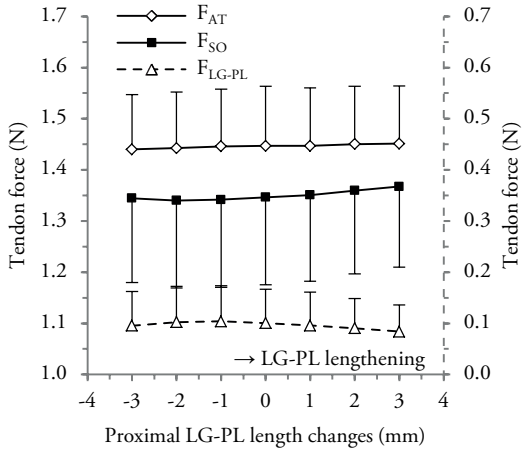

Figure 6.2. Effects of LG-PL length on active force exerted at the distal tendons of SO (■, left *y*-axis) and LG-PL (Δ, right *y*-axis) and on the calculated active Achilles tendon force (◊, left *y*-axis) during SO excitation only. Means±SD are shown (n = 5).

Discussion

We found epimuscular myofascial force transmission between ankle plantar-flexors for physiological ranges of muscle displacements. In agreement with our hypothesis, mechanical interaction between muscles was enhanced by increased levels of muscle activation. However, the magnitudes of myofascial forces were limited and only partially reflected in the Achilles tendon force.

Effects of SO&LG activation on the extent of intermuscular mechanical interaction

As pointed out earlier (Herbert *et al.*, 2008), maximal excitation of SO without excitation of its synergists is rarely seen during motor tasks, which questions the physiological relevance of such an excitation protocol. Although selective activation of ankle plantar-flexion muscles has been found during prolonged, low-level static plantar-flexion in humans (Tamaki *et al.*, 2011) and during paw-shakes in cats (Smith *et al.*, 1980), these muscles are indeed co-activated during a variety of other activities. Co-activation of SO, LG and medial gastrocnemius (MG) has been reported during human gait (Ishikawa *et al.*, 2005), cycling (Wakeling, 2009) and (sub)maximal isotonic, isometric and isokinetic heel rising tasks (Ball & Scurr, 2015), as well as in rat (Roy *et al.*, 1991) and cat (Maas *et al.*, 2009; Markin *et al.*, 2012) locomotion. More specifically, near-maximal SO activation combined with submaximal MG activation has been found in upslope walking in rats (Roy *et al.*, 1991) and cats (Pierotti *et al.*, 1989). This indicates that the relative co-activation levels of synergistic muscles used in the present study can be considered physiological.

A previous study reported that, during maximal excitation of SO, LG and PL muscles, proximal lengthening the LG-PL complex increased the force exerted at the distal SO tendon by approximately 10% (Bernabei *et al.*, 2015). In the present study, a lower increase in distal SO tendon force with LG co-activated was found (4.3%). This can be explained by the fact that, in the present study, PL was passive and LG was excited only partially (see Materials & Methods). Nonetheless, the effects of LG-PL length on distal SO tendon force during SO&LG excitation were more pronounced than during excitation of SO only, indicating increased intermuscular mechanical interaction. The results of the present and previous study (Bernabei *et al.*, 2015) combined confirm that the extent of intermuscular mechanical interaction is dependent on the amount of synergistic muscle force, as hypothesized earlier (Maas & Sandercock, 2008): higher levels of synergistic muscle force resulted in an increased extent of mechanical interaction.

The increased interaction during SO&LG excitation can be explained by two potential mechanisms: (1) with increasing LG-PL length, more LG muscle force is exerted on the distal SO tendon; (2) with increasing LG-PL length, more force produced by SO muscle fibers is exerted on its own distal tendon instead of on the distal LG-PL tendon. We found that, during separate SO excitation, part of the

active force generated by SO muscle was exerted at the distal LG-PL tendon (Fig. 6.2). Also during separate excitation of LG, additional forces were observed at the distal SO tendon but these were much lower (Fig. 6.1A). This indicates that, during separate SO and LG excitation, the net orientation of distally located connective tissue linkages was such that force transmission occurred mainly from SO to LG-PL and not vice versa. Although no information was available about the orientation of connective tissue linkages during SO&LG excitation, the second mechanism is the most probable explanation of the increased interaction during SO&LG excitation.

Intermuscular force transmission during separate SO excitation

During separate SO excitation, additional forces (on average 0.1 N) on the LG-PL tendon were measured. However, proximal length changes of the LG-PL complex (i.e. resulting in changes in the orientation of mainly proximal located linkages) did only minimally affect this additional force. This suggests that proximally located connections at the interface between SO and LG-PL are slack or on the toe region of the stress–strain curve (Sandercock & Maas, 2009), and that the proximally located connections are more compliant than distally located connections, or oriented such that they allow LG-PL muscle bellies to move proximally with minimal resistance.

In the present study, we found a limited change (2.0%, Fig. 6.2) in active F_{SO} during SO excitation if the LG-PL complex was lengthened proximally. The change of the calculated F_{AT} was even smaller (0.8%). In an intact system, this would imply limited changes in the force exerted at the calcaneus and, therefore, also limited changes in the exerted joint moment. The results of the present study can explain the absence of effects of knee angle (i.e. proximal lengthening GA and PL) on the ankle moment exerted by SO in cats (Maas & Sandercock, 2008) and rats (Tijs *et al.*, 2015*a*): proximal LG-PL lengthening minimally affects SO force output, and, although force is transmitted between these muscles, both muscles transmit force to the calcaneus via the shared Achilles tendon.

Although the mechanical relevance of intermuscular connections between triceps surae muscles may be limited at the level of the ankle joint, epimuscular myofascial force transmission may distribute stresses and strains over multiple muscles and tendons, which might reduce local stresses (Bojsen-Møller *et al.*, 2010). Additionally, intermuscular force transmission may result in local length changes

within a length-restrained muscle. Muscle spindles within these length-restrained muscles may detect such deformations, thereby affecting sensory encoding (Smilde *et al.*, 2014).

Conclusions

We conclude that for physiological muscle lengths and relative positions (i) epimuscular myofascial force transmission is present between rat ankle plantar-flexion muscles; (ii) the extent of such intermuscular mechanical interaction is dependent on the level of muscle activation and (iii) epimuscular myofascial force transmission is only partially reflected in the Achilles tendon force. Due to the fact that the tendons of these muscles merge into a shared Achilles tendon, the mechanical relevance of epimuscular myofascial force transmission between ankle plantar-flexors at the joint level is limited.